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Molecular, Cytogenetic, and Clinical Investigations of Prader-Willi Syndrome Patients

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Summary

Thirty-seven patients presenting features of the Prader-Willi syndrome (PWS) have been examined using cytogenetic and molecular techniques. Clinical evaluation showed that 29 of these patients fulfilled diagnostic criteria for PWS. A deletion of the 15q11.2-q12 region could be identified molecularly in 21 of these cases, including several cases where the cytogenetics results were inconclusive. One clinically typical patient is deleted at only two of five loci normally included in a PWS deletion. A patient carrying a de novo 13;X translocation was not deleted for the molecular markers tested but was clinically considered to be "atypical" PWS. In addition, five cases of maternal heterodisomy and two of isodisomy for 15q11-q13 were observed. All of the eight patients who did not fulfill clinical diagnosis of PWS showed normal maternal and paternal inheritance of chromosome 15 markers; however, one of these carried a ring-15 chromosome. A comparison of clinical features between deletion patients and disomy patients shows no significant differences between the two groups. The parental ages at birth of disomic patients were significantly higher than those for deletion patients. As all typical PWS cases showed either a deletion or disomy of 15q11.2-q12, molecular examination should provide a reliable diagnostic tool. As the disomy patients do not show either any additional or more severe features than typical deletion patients do, it is likely that there is only one imprinted region on chromosome 15 (within 15q11.2-q12).

Introduction

The Prader-(Labhart-)Willi syndrome (PWS) was first described in 1956 and was defined, on the basis of the findings present in nine original patients, to include grossly diminished fetal activity, severe infant hypotonia, feeding problems in infancy, hypogonadism and hypogenitalism, retarded bone age and short stature, small hands and feet, delayed mental and psychomotor development, characteristic facies, mental retardation, onset of gross obesity (because of insatiable hunger) in early childhood, behavior problems, and a tendency to develop diabetes in adolescence (Prader et al. 1956). These features, however, can be quite variable among individual patients. PWS has an incidence of approximately 1/10,000–1/30,000 live births (Cassidy 1984).

The association between an interstitial deletion of part of chromosome 15 and PWS was shown in 1981 (Ledbetter et al. 1981, 1982). Interstitial deletions of the proximal region of 15q are reported in about 60% of PWS patients, while 37% are apparently normal cytogenetically, and 3.6% show other chromosomal anomalies involving chromosome 15 (Ledbetter et al. 1987). Duplications, unbalanced and apparently balanced translocations, or rearrangements have been reported (for review, see Butler 1990). The development of molecular probes for 15q11.1-q12 has enabled the detection of deletions in patients, without requiring cytogenetic techniques (Donlon et al. 1986; Donlon 1988; Nicholls et al. 1989*b*; Tantravahi et al. 1989; Buiting et al. 1990).

Both molecular and cytogenetic studies have shown that the deleted chromosome in PWS patients always

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originates from the father (Butler et al. 1986; Nicholls et al. 1989*b*). The 15q11-q13 region is also deleted in most Angelman syndrome (AS) patients (Kaplan et al. 1987; Magenis et al. 1987; Donlon 1988; Knoll et al. 1989; Pembrey et al. 1989; Williams et al. 1989, 1990), with the deleted region originating exclusively on the maternal chromosome 15 (Knoll et al. 1989; Magenis et al. 1990; Williams et al. 1990). AS presents a clinical picture distinct from that of PWS, although both include mental retardation (but to a different degree) and hypopigmentation and sometimes share hypotonia (also to varying degrees) and hypogenitalism. Epilepsy, a common feature of AS, is sometimes found in PWS patients. Cytogenetic deletions of 15q11-q13 have also been reported in patients presenting features not consistent with either PWS or AS (Schwartz et al. 1985; Kaplan et al. 1987; Reynolds 1987). However, a gene for the presumably autosomal recessive Cohen syndrome, which is also characterized by hypotonia, obesity, and mental retardation, has been excluded from the 15q11-q12 region (Kondo et al. 1990).

An interesting insight into the genetics of PWS was the discovery of two cases of PWS who displayed uniparental disomy—i.e., the inheritance of two copies of a chromosome (or of part of a chromosome) from one parent and none from the other parent (Nicholls et al. 1989*a*). These cases showed maternal heterodisomy for chromosome 15q11-q13, i.e., the inheritance of two different chromosome 15's both derived from the mother. Paternal disomy of chromosome 15—i.e., the inheritance of two paternal and no maternal chromosome 15's—has also been observed in AS (Malcolm et al. 1991). These results support the hypothesis that (*a*) PWS may be caused by the lack of a specific paternally inherited gene(s) within 15q11-q13 (with inactivation of the corresponding maternal gene(s) through imprinting) and (*b*) AS may result from the lack of maternally inherited gene(s) (with the presence of only paternally inactive, imprinted gene(s)) within this same segment.

Few differences between PWS patients with or without a deletion have been reported to date. It has been observed that PWS patients with a visible deletion have higher intelligence (Butler et al. 1986) and more frequent hypopigmentation of skin, hair, and iris (Butler et al. 1986; Wiesner et al. 1987), relative to those patients without a visible deletion. Patients with relatively large deletions as determined by cytogenetic studies are reported to have more severe manifestations of the PWS phenotype (Magenis et al. 1987). However, because of the difficulty of definitively identifying deletion- and nondeletion-carrying patients cytogenetically, misclassification of patients can obscure differences between the two groups. The use of molecular probes should help to clarify the clinical features between these groups, as well as to identify features peculiar to disomy patients.

In the present study, 37 patients were examined both cytogenetically using G-banding techniques and molecularly by RFLP analysis and densitometry of 15q11-q13 markers. The patients were referred for genetic studies because of a suspicion of PWS. Several patients whose diagnosis did not clearly fit PWS but who displayed some similar features were also included in this group. Examination of such individuals is important for determining the range of clinical features which could potentially be associated with a deletion or uniparental disomy of chromosome 15. The frequency at which uniparental disomy occurs in PWS patients, insight into the source of the meiotic error, and identification features or prognoses that might distinguish such patients from those carrying a chromosome 15q11-q13 deletion are discussed. Those patients who were found to have two normal chromosome 15's, one from each parent, were examined to answer the question: Are there patients who truly display the PWS phenotype yet have inherited an apparently intact chromosome 15 from their father?

Patients and Methods

Patients

Patients with the diagnosis or suspicion of PWS were referred, by independent medical doctors, either to the Department of Pediatrics or to the Institute of Medical Genetics at the University of Zürich; or, in several cases, the initial diagnosis was made by one of us (A.P., A.S., or A.B.). Cytogenetic and molecular studies were predominantly completed without prior knowledge of the clinical features. Conversely, all of these patients were reevaluated clinically by A.P. and/or A.S., without knowledge of the molecular genetic results, in order to identify (*a*) which patients did or did not fulfill diagnostic criteria for PWS and (*b*) any atypical features, such as less severe mental retardation or a not completely typical history (i.e., normal intrauterine activity, lack of feeding problems in infancy, etc.).

Clinical evaluation was done according to the following criteria: every patient was screened for the clinical findings listed in table 1. Only one of these major

Table 1

PWS Diagnosis Criteria

Period	Symptoms
Pregnancy.....	Diminished fetal movements (as compared with previous pregnancy)
Neonatal period....	Severe hypotonia; feeding difficulties; hypogenitalism in boys
Early childhood....	Retarded motor/mental development; hypotonia; increased appetite; slender hands and fingers, short feet; characteristic facies (hypotonic, down-slanting palpebral fissures, almond-shaped eyes, and down-turned corners of mouth)
Late childhood	Obesity with knock-knees; hypogenitalism/cryptorchidism; diminished puberty; predicted adult height below target height

characteristics of PWS was allowed to be lacking in a patient considered to have the full pattern of PWS (reduced intrauterine activity was not scored in first-born children because the mothers were not able to compare their pregnancy with previous ones). If more than three major features were absent, the patient was not considered to have PWS. With two or three features lacking, he or she was considered to have "atypical" PWS. Features were only considered if they could be expected to be present; hence, obesity was disregarded if the patient was younger than 2 years old, hypogenitalism was not scored in females, diminished puberty was not considered in children, etc. In order to evaluate obesity, we considered both weight percentile and skinfold thickness. Patients with increased skinfold but grossly diminished muscle mass were not always above the 95th percentile in weight but were still considered to be obese.

Twenty-nine patients were originally classified as having PWS, and seven patients (PW4, PW29, PW54–PW56, PW64, PW70, and PW79) did not initially fulfill diagnostic criteria for PWS but showed some similar features (see table 2). One patient (PW66), however, was initially classified as atypical PWS but during the course of our study underwent a normal puberty. This is enough to disallow this patient from being considered to fulfill criteria for PWS. Patient PW54 was only 8 mo old when the diagnosis of non-PWS was made, but, by 1 year of age, developmental changes allowed this patient to be considered typical of PWS. Reexamination of this patient was done as a direct consequence of the molecular results; however, the clinical changes were clear enough that the altered diagnosis was not biased.

The following clinical features of patients were compared in order to find features that distinguish the deletion and nondeletion patients: history of intrauterine activity in the mother during pregnancy; delivery term, birth weight and length at birth; history of severe neonatal hypotonia; duration of gavage feeding; time of first free sitting and steps; age at hyperphagia onset; percentile, at examination, of weight, height, head circumference, and hand and foot lengths; presence and extent of fair hair (relative to parents); scars due to compulsive scratching; indifference to pain; strabismus; hypotonia; mental/motor retardation; narrow forehead; frontal upsweep, epicanthal folds, nonhorizontal slant of palpebral fissures; hypogenitalism; cryptorchidism; striae; diabetes; and knock-knees. Data were incomplete for some individuals. All the patients come from Switzerland, Italy, or Germany and were 1–26 years of age.

Cytogenetics

Peripheral blood lymphocytes from patients and parents were cultured using methods that increase the frequency of late-prophase-to-early-metaphase chromosomes (Yunis 1976). Complete karyotype analysis of G-banded chromosomes (trypsin-Giemsa staining using standard techniques) was performed with attention to the possibility of a chromosome 15 deletion. Blind analysis of karyotypes was done when possible; that is, the identity of who were parents or patients was withheld from the cytogeneticist performing the analysis. High-resolution banding was not possible in every case. For cases where the presence or absence of a deletion was difficult to determine, the cytogenetic results were reported as nondeletion but are indicated in table 3 as being uncertain.

DNA Analysis

Genomic DNA was isolated from EDTA-anticoagulated blood (Baas et al. 1984). Restriction-enzyme digestion, electrophoresis, and DNA blotting (Southern 1975) onto either Hybond-N (Amersham) or nitrocellulose (Schleicher and Schüll) filters were performed according to standard procedures. Membranes were prehybridized (50% formamide, 10 × Denhardt's solution [= 0.2% each of BSA, polyvinylpyrrolidone 360, and Ficoll 400], 0.1 mg denatured salmon sperm/ml, 0.1% SDS, 20 mM sodium phosphate, and 0.9 M NaCl) at 42°C for 6–16 h. Probes were labeled with ^{32}P by nick-translation using a com-

Table 2

Clinical Features of All Patients

Patient (sex, age in years)	Weight (%)	Height (%)	Parental Age[a] (years)		Weight at Birth (g)	Length at Birth (cm)	Term (wk)	Status of Clinical Feature[b]									
			Mother	Father				RIA	GF	NH	HG	CO	ST	CF	FH	NF	IP
Nondeletion, nondisomy patients:																	
PW4 (M, 15)	>97	<3	28	36	2,350	44	−2	−	0	Mild	−	−					+
PW29 (F, 3)	>97	50	37	33	3,740	51	−1	+	0	−		NA	+	−	−	+	+
PW46 (F, 12)	50–75	3–10	26	29	2,900	48	−2	+ +		+		NA	−	+	+	−	
PW55 (F, 13)	<3	<3			4,300	52	0	−	+	+	−	NA	−	−	−	−	
PW56 (F, 8)	>97	<3	21	28	2,790	51	0		0	−		NA			−		
PW64 (F, 14)	25	<3	34	31	2,900	47	−1	+	0	−		NA	−	−	−	−	
PW66 (F, 12)	25–50	<3	31	35				+	+	+	−	NA	+				
PW70 (M, 26)	>97	50	28	31	3,300		−2	−	0	−	+	+	+	−		−	−
PW79 (M, 10)	90–97	10–25			2,400		−1	−	−	−	+	+	+	−	−	+	−
Disomy patients:																	
PW1 (M, 26)	>97	<3	40	44	4,200		0	+ +		+	+	+		+		+	+
PW9 (M, 17)	>97	3	41	40	2,800		0	−	0	+	+	−	+	+	−	+	+
PW23 (M, 3)	90–97	50–75	22	23	2,800	51	2	−	4	Mild	+	+	+	+	+	−	
PW54 (M, <1)	<3	<3	39	45	1,440	40	−8	+	6	+	−	−				+	−
PW63 (M, 1)	90	75	35	35	3,200		−1	+ +	4	+	+	+	−	+	+	+	
PW77 (M, 4)	>97	25	40	48	2,500	51	0	+	3	+	+	+	+	+	+	+	
PW81 (M, 3)			32	36	3,010	49	0	+ +	0	+	+	+	−	+		+	+
Deletion patients:																	
PW7 (F, 19)	<3	<3	32	34	2,250	45	−5	+ +	8	+	−	NA	+	+	+	+	
PW12 (M, 4)	>97	75–90			2,690	47	−4	+	0		+	+	−			+	
PW15 (F, 14)	75–90	<3	24	31	2,380		0		4	+ +	+	NA	+	+			+
PW18 (M, 5)	>97	50	26	27	2,900	50	2	+ +	+	+	+	+		+		+	+
PW24 (M, 14)	>97	3–10	20	23	2,650	49	0	+ +	0	+	+	+	+	+	+	+	−
PW26 (F, 13)	90–97	<3	28	30	2,350	47	0	+	12	+ +	+	NA	−	+	+	+	
PW37 (F, 18)	50	<3	21	21	2,200		−1	+	+	+ +	+	NA	+	+			
PW38 (F, 21)	97	50	32	34	2,700	48	4			+	+	NA				+	
PW43 (F, 10)	>97	<3	20		2,600	47	−3	+		+		NA		+	+		
PW47 (F, 3)	>97	10	35	33			7	+		+	+	NA	+	+		+	
PW48 (M, 12)	>97	<3	32	28	3,100	51	4	+ +	4	+	+	+	+	+	+		+
PW49 (F, 5)	90–97	3–10	20				3	+ +	8	+		NA		+	+	+	
PW51 (F, 16)	50–75	<3	27	32	2,350	46		+	0	+	+	NA	+	+	+		+
PW52 (M, 8)	90–97	3	22	24	2,960	50		+ +	4	+ +	+	+	+	+	+	+	+
PW61 (F, 22)	>97	<3	35	33	2,650	50	0	−	4	+	+	NA	+	+	+	+	+
PW62 (M, 19)	>97	<3	29	36	3,240		0	+ +	0	+	+	+		+	+	+	+
PW65 (M, 10)	>97	50	27	33	2,800		2	+ +	5	+	+	+	+	+	+	+	+
PW69 (F, 20)	>97		35	36	2,950	49	0	+ +	10	+		NA	−	+	+	+	+
PW76 (M, 9)	>97	50	29	33	2,610	53	0	+	+	+	+	+	+	+	−	+	
PW78 (F, 18)	3–10	<3	26		1,800		−4	−	12	+		NA	+	+		+	
PW93 (M, 10)	90	3–10	23	25	2,630	48	1	+ +	32	+	+	+	+	+	+	+	−

[a] At birth of patient.

[b] RIA = reduced intrauterine activity; GF = weeks of gavage feeding; NH = severe neonatal hypotonia; HG = hypogenitalism; CO = cryptorchidism; ST = strabismus; CF = characteristic facies; FH = fair (relative to parents); NF = narrow forehead; and IP = indifference to pain. + = Present; + + = severe; − = absent; NA = not available.

Table 3

Molecular, Clinical, and Cytogenic Results

Patients	Gene Dosage Estimate For[a]						Clinical Diagnosis	Molecular Classification	Cytogenetic Diagnosis
	pIR4-3R	p189-1	p34	p3-21	pIR10-1	pCMW-1			
Nondeletion, nondisomy patients:									
PW4.....	+	2.1	het	1.8[b]	+	het	Non-PWS	Maternal and paternal inheritance[c]	46XY
PW29....	het	het	het	+	het	+	Non-PWS	Maternal and paternal inheritance	46XX
PW46....	het	1.7	+	het	NT	het	Atypical PWS	Maternal and paternal inheritance	46XX t(X;13)
PW55....	het	het	het	1.9	+	+	Non-PWS	Maternal and paternal inheritance[c]	46XX
PW56....	+	het	+	1.9	het	+	Non-PWS	Maternal and paternal inheritance[c]	46XX
PW64....	+	het	+	2.0	+	het	Non-PWS	Maternal and paternal inheritance	46XX
PW66....	het	2.1	+	2.1	+	het	Non-PWS	Maternal and paternal inheritance	46XX ring 15
PW70....	het	het	+	1.7	+	+	Non-PWS	Maternal and paternal inheritance	46XY
PW79....	+	het	+	2.1	+	het	Non-PWS	Maternal and paternal inheritance	Not done
Disomy patients:									
PW1.....	het	1.7	+	1.7	het	+	PWS	Maternal heterodisomy	46XY[d]
PW9.....	+	2.0[e]	+	2.1[b]	+	+	PWS	Maternal isodisomy	46XY[d]
PW23....	+	1.7[b]	+	2.2[e]	+	+	PWS	Maternal isodisomy	46XY
PW54....	het	het	+	2.0[b]	+	het	PWS	Maternal heterodisomy	46XY
PW63....	+	het	+	het	+	het	PWS	Maternal heterodisomy	46XY
PW77....	+	1.6[b]	+	2.0[b]	het	het	PWS	Maternal heterodisomy	46XY
PW81....	+	2.5	+	het	+	het	PWS	Maternal heterodisomy	46XY
Deletion patients:									
PW7.....	–	.8	–	.8[b]	–	NT	PWS	Deletion	46XX del (15q12)
PW12....	–	1.5	–	1.3[e]	–	het	PWS	Deletion	46XY[d]
PW15....	–	1.1	–	1.4[b]	–	NT	PWS	Deletion	46XX del (15q12)-paternal
PW18....	–	1.0	–	1.0[b]	–	het	PWS	Deletion (paternal)	46XY del (15q12)
PW24....	–	1.3	–	.8[b]	–	het	PWS	Deletion	45XY del (15q12)
PW26....	–	–	–	.5[b]	–	NT	PWS	Deletion (paternal)	46XX del (15q12)
PW37....	NT	.9	–	.8[b]	–	NT	PWS	Deletion	46XX[d]
PW38....	NT	.9	–	.9	–	NT	PWS	Deletion	46XX[d]
PW43....	NT	.8	–	.9	–	NT	PWS	Deletion	46XX del (15q12)
PW47....	–	1.0[b]	–	.8[b]	–	het	PWS	Deletion (paternal)	46XX[d]
PW48....	–	.7	–	1.0[b]	–	+	PWS	Deletion (paternal)	46XY del (15q12)
PW49....	–	.9	–	.9[b]	–	NT	PWS	Deletion (paternal)	46XX[d]
PW51....	–	1.0	–	1.0	–	+	PWS	Deletion (paternal)	45XX t(15;20) (q13;q13)
PW52....	–	.6	–	.8[b]	–	NT	PWS	Deletion (paternal)	46XY del (15q12)
PW61....	–	.8[b]	–	1.1[e]	–	NT	PWS	Deletion (paternal)	46XX del (15q12)
PW62....	–	.8	–	.7[b]	–	NT	PWS	Deletion	46XY del (15q12)
PW65....	–	.7	NT	1.0[b]	NT	het	PWS	Deletion (paternal)	46XY del (15q12)
PW69....	–	1.4[e]	–	1.5[b]	–	+	PWS	Deletion (paternal)	46XX del (15q12)
PW76....	–	.7	–	.7	–	–	PWS	Deletion	45XY t(9;15) (p24.3;q13)
PW78....	NT	1.0[e]	–	1.3	–	+	PWS	Deletion	46XX del (15q12)
PW93....	het	het	+	.9	–	–	PWS	Deletion	46XY del (15q12)

[a] For pIR4-3R, p34, pIR10-1, and pCMW-1, dosage was estimated visually; for p189-1 and p3-21, dosage was estimated by densitometry. + = No deletion observed; – = deletion present; het = heterozygous; NT = not typed.

[b] Average of readings from two separate Southern blots.

[c] Paternal inheritance could only be shown with the telomeric probe pMS620.

[d] Insufficient resolution makes cytogenetic diagnosis uncertain.

[e] Average of readings from three separate Southern blots.

mercial kit from Amersham. Hybridization took place at 42°C for 16–20 h.

Molecular studies on all patients and on all available parents were undertaken using the polymorphic probes in the PWS region (15q11-q13): pIR39 (D15S18), pML34 (D15S9); p3-21 (D15S10), pIR4-3 (D15S11), pIR10-1 (D15S12), and p189-1 (D15S13) (Donlon et al. 1986; Nicholls et al. 1989*b*; Tantravahi et al. 1989). Two probes (p189-1 and p3-21) were used for densitometric analysis in order to determine the presence or absence of deletions in patients. A probe from chromosome 7 (pJ3.11), hybridizing to a constant 6-kb *Taq*-digested fragment, was used as an internal control for dosage comparison (this probe also recognizes a rare 3.2-kb allele, which was found in a few families and was accounted for accordingly). In addition, three probes—pCMW-1 (D15S24) (Rich 1988), recognizing a VNTR located just distal to the PWS region; pMS-620 (Armour et al. 1990), recognizing a VNTR in the telomeric region of chromosome 15; and pMS1-14 (D15S1) (Barker et al. 1984), located to 15q14-q22—were used to investigate uniparental disomy. The loci recognized by pCMW-1 and pMS1-14 are separated by about 0.26 cM, with pCMW-1 located nearer to the centromere (Nakamura et al. 1988). Nonpaternity was excluded in all families by using Jeffreys' hypervariable probe 33.11 (Jeffreys et al. 1985).

Quantitative analysis of autoradiographs (Fuji X-ray film) was performed on a Bio Image (Milli Gen/Biosearch) digital image-processing instrument and Visage Gel Analysis software (Millipore). Data were recorded as a ratio of the chromosome 15 probe peak area to the chromosome 7 probe peak area (corrected for background levels). Because normally the two normal parents were run along with every patient, a calibration curve of the peak area ratios (PARs) versus optical density (OD) could be estimated for each gel separately, by using homozygous controls to estimate the two-copy ratio and by using heterozygous controls to estimate the single-copy ratios. Although the relationship between dosage and peak area is not strictly linear, for the purposes here, where we are only testing whether individuals have a single or double dose at a test locus, it is reasonable to convert PARs into band dosage values by assuming a linear relationship and by using the estimated double- and single-copy PARs as reference points. The majority of cases were repeated for probes p3-21 and/or p189-1 on a separate Southern blot to verify the presence or absence of a deletion.

Results

Deletion Analysis

By means of broad-slot (1-cm) gels and simultaneous hybridization of a non–chromosome 15 constant marker, deletions in patients' DNA could easily be observed visually when compared with heterozygous and homozygous control samples on the same gel (fig. 1). Densitometric analysis of p189-1 and p3-21 hybridization confirmed that 21 patients carried deletions within 15q11.2-q12. One patient (PW93) was deleted for p3-21 (fig. 1, *left*) but was heterozygous for the loci detected by probes p189-1 and pIR4-3R. A summary of densitometric results is given in table 3. All patients showing a 15q11.2-q12 deletion detectable by these two probes were, independently from these results, judged to fulfill diagnostic criteria for PWS.

In order to determine the parental origin of the deletion in those cases who were shown to be 15q11.2-q12 deleted and for whom both parents were available for study (N = 17), additional polymorphic probes were used. Noninheritance of a paternal allele could be identified in 10 deletion cases (data not shown). In one additional case the deletion could be determined, by cytogenetic means, to be paternal in origin. No cases were observed who showed noninheritance of a maternal allele. The remaining six cases were uninformative for the markers examined.

Simple deletions of the 15q12 band were observed by cytogenetic methods in 14 of the deletion cases (table 3). In addition, an unbalanced t(15;20) translocation was observed in PW51, and an unbalanced t(9;15) was seen in PW76, with a deletion extending from 15q13 to the centromere in both cases. In five cases, a molecular deletion was seen when a cytogenetic one was not; however, in all of these cases the cytogenetic results were of suboptimal quality, and these patients were not clearly nondeletion either.

Although densitometric studies were not completed for probes pIR10-1, pIR4-3R, and p34, approximately equal amounts of DNA were loaded in each lane, and band strength was compared to that of normal control samples run on the same gel. This method should not be relied on for diagnostic purposes, as it is not possible to get exactly equal amounts of DNA in each well; however, the results were consistent with the results from probes p189-1 and p3-21. No patients who had not already been shown to be deleted for p189-1 and/or p3-21 were observed to be deleted for any of these probes. There was only one case (PW93)

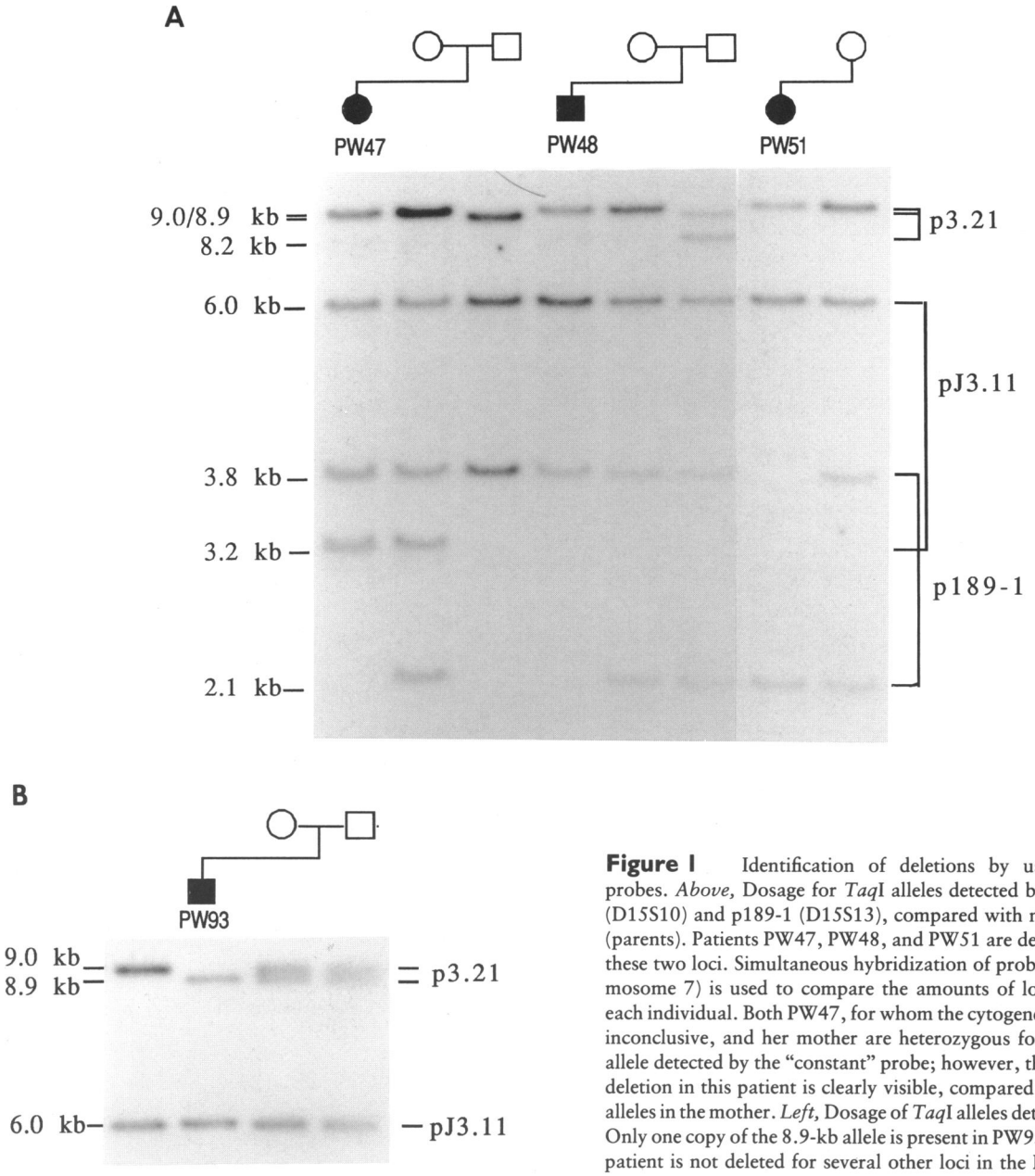

Figure 1 Identification of deletions by using molecular probes. *Above,* Dosage for *Taq*I alleles detected by probes p3-21 (D15S10) and p189-1 (D15S13), compared with normal controls (parents). Patients PW47, PW48, and PW51 are deleted at both of these two loci. Simultaneous hybridization of probe pJ3.11 (chromosome 7) is used to compare the amounts of loaded DNA for each individual. Both PW47, for whom the cytogenetic results were inconclusive, and her mother are heterozygous for a rare 3.2-kb allele detected by the "constant" probe; however, the presence of a deletion in this patient is clearly visible, compared with dosage of alleles in the mother. *Left,* Dosage of *Taq*I alleles detected by p3-21. Only one copy of the 8.9-kb allele is present in PW93, although this patient is not deleted for several other loci in the PWS region.

who appeared to be deleted for some but not all of the probes p189-1, p3-21, pIR4-3R, pIR10-1, and p34. This patient, who is very typical of PWS clinically, was heterozygous for pIR4-3R and p189-1 (with one allele from each parent) but deleted for p3-21. The patient appeared to have two copies of the locus detected by p34 and to have a deletion for pIR10-1 as well. A deletion was observed in this patient by cytogenetics, and so, although the molecular results exclude the possibility that the region including and centromeric to the loci detected by pIR4-3R and p189-1 is critical to the PWS phenotype, there is still probably a relatively large deletion in this patient.

Some deletion patients were also examined with pCMW-1. Many were heterozygous for this probe and therefore not deleted; however, a deletion for this probe was seen in PW76, a carrier of an unbalanced t(9;15). The other unbalanced-translocation carrier

Table 4

Molecular Results in Disomy Patients

	Alleles Occurring at[a]								
	15q11.2-12						15q13:	15q14-21:	Telomere:
Centromere	p39	pIR4-3R	p189-1	p34	p3-21	pIR10-1	pCMV-1	pMS1-14	pMS-620
PW1[b]	12	12	11	11	11	23	aa	12	aa
Mother	12	12	11	11	11	23	aa	12	ab
Father	22	12	12	11	11	23	bc	22	cd
PW9[c]	22	11	11	11	11	33	bb	12	ab
Mother	22	11	11	11	11	23	bc	12	ab
Father	12	22	22	12	12	12	ab	12	cd
PW23[d]	22	22	11	11	11	22	aa	12	ab
Mother	22	12	11	11	13	23	aa	12	ab
Father	22	22	12	22	12	12	ac	11	cd
PW54	12	12	12	11	11	22	ab	12	ab
Mother	12	12	12	11	11	22	ab	12	ab
Father	11	12	12	11	13	22	bb	22	cc
PW63	12	22	12	11	13	11	ab	12	ab
Mother	12	22	12	11	13	11			ab
Father	12	11	11	11	11	11			bc
PW77[e]	12	22	22	22	11	12	ab	22	aa
Mother	12	22	22	22	11	12	ab	22	ab
Father	22	11	22	22	12	11		22	cd
PW81	12	11	22	11	12	22	ab	22	ab
Mother	12	11	22	11	12	22	ab	22	ab
Father	22	12	11	12	11	22	bc	22	bc

[a] Alleles at the various loci are as follows (codes used in are indicated in parentheses): p39 detects 9.0-kb (1) and 8.5-kb (2) *BglII* alleles; pIR4-3R detects 1.2-kb (1) and 1.0-kb (2) *RsaI* alleles; p34 detects 6.5-kb (1) and 6.3-kb (2) *ScaI* alleles; p189-1 detects 3.8-kb (1) and 2.0-kb (2) *TaqI* alleles; p3-21 detects 9.0-kb (1), 8.9-kb (2), and 8.2-kb (3) *TaqI* alleles; pIR10-1 recognizes 17.5-kb (1), 16-kb (2), and 12.5-kb (3) *ScaI* alleles; pCMW-1 and pMS-620 detect VNTRs by using *TaqI* and are coded for in each family separately; and pMS1-14 detects 12-kb (1) and 4.3-kb (2) *MspI* alleles.

[b] Recombination observed between loci recognized by probes pMS1-14 and pMS-620.

[c] Recombination observed between loci recognized by probes pCMW-1 and pMS1-14.

[d] Recombination observed either between loci recognized by probes pIR10-1 and pCMW-1 or between loci recognized by probes pCMW-1 and pMS1-14.

[e] Recombination observed between loci recognized by probes pCMW-1 and pMS620.

(PW51) was not deleted for this probe, although the deletion in both patients seems to extend from 15q13 to the centromere.

Disomy Analysis

Of those 16 (PWS and non-PWS) patients in whom no deletion was observed, seven could be shown to exhibit uniparental (maternal) disomy (molecular results are summarized in tables 3 and 4). In patient PW1, only maternal inheritance is observed both for the VNTR recognized by probe pCMW-1 (fig. 2*A*) and at the telomeric VNTR recognized by probe pMS-620. At the locus recognized by pCMW-1, both mother and child are homozygous for the same allele; however, at loci recognized by pIR4-3R (fig. 2*B*) and pIR10-1 (not shown), the patient is heterozygous and identical to his mother. Patient PW1 is thus most likely heterodisomic for the PWS region. Similarly, patient PW63 is heterozygous and identical to his mother, for both p189-1 and p3-21, and no paternal inheritance could be seen with probe pIR4-3R (data not shown). PW77 does not inherit paternal alleles identified by pIR4-3R and is heterozygous for alleles detected by pIR10-1 (data not shown). PW81 shows noninheri-

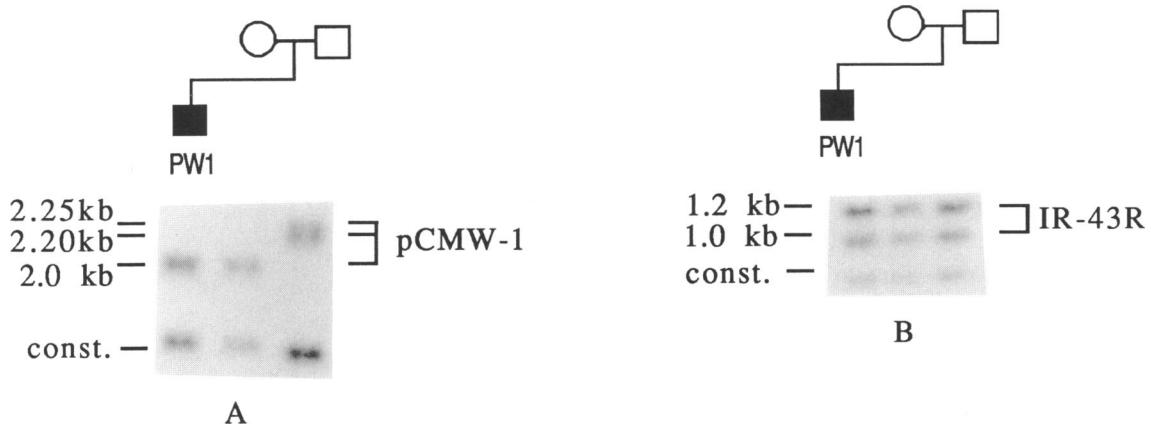

Figure 2 Molecular results supportive of maternal heterodisomy in PW1. *A*, pCMW-1 (D15S24), for which only maternal inheritance is seen. *B*, PW1, heterozygous for 1.2-kb and 1.0-kb alleles recognized by pIR4–3R (D15S11). PW1 is identical to his mother, although a paternal contribution cannot be excluded.

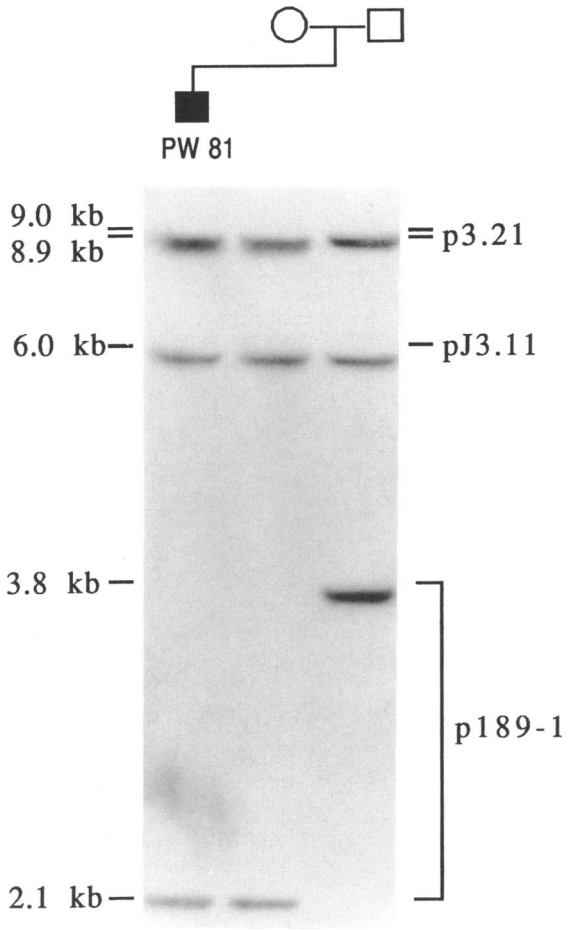

Figure 3 Molecular results supportive of maternal heterodisomy in PW81. The patient did not inherit the paternal 3.8-kb allele recognized by p189-1 (D15S13) and is heterozygous and identical to his mother at the locus recognized by p3-21 (D15S10) (this result was verified by running a separate Southern blot with better separation of the two p3-21 alleles).

tance of paternal alleles when probe p189-1 is used, and he is heterozygous for p3-21 (fig. 2). PW1, PW77, and PW81 have typical PWS features which are indistinguishable from those of a classical PWS deletion patient. PW63 is only 1½ years old but, as yet, also shows typical PWS features

In addition, PW54 is consistent with maternal disomy for all probes in the PWS region and, by the telomeric probe pMS-620, shows noninheritance of paternal alleles. This patient, who was born 2 mo prematurely and also had problems associated with prematurity, was originally not considered to fulfill diagnostic criteria for PWS. He was only 8 mo old when examined, and a muscle atrophy or dystrophy was considered the most likely diagnosis at that time. Reevaluation of this patient subsequent to the molecular findings indicated that several features previously masked by prematurity, such as developmental/mental retardation and typical facial features, are now (at 12 mo) apparent in this patient. The only PWS diagnostic feature not present is hypogenitalism, and he is thus now considered to be typical of PWS.

Patients PW9 and PW23 are homozygous at all loci tested in the PWS region, including loci for which the mothers are heterozygous. No inheritance of paternal alleles at loci recognized by probes p189-1, pIR4-3R, and pIR10-1 (fig. 4) can be seen in PW9, and by p34

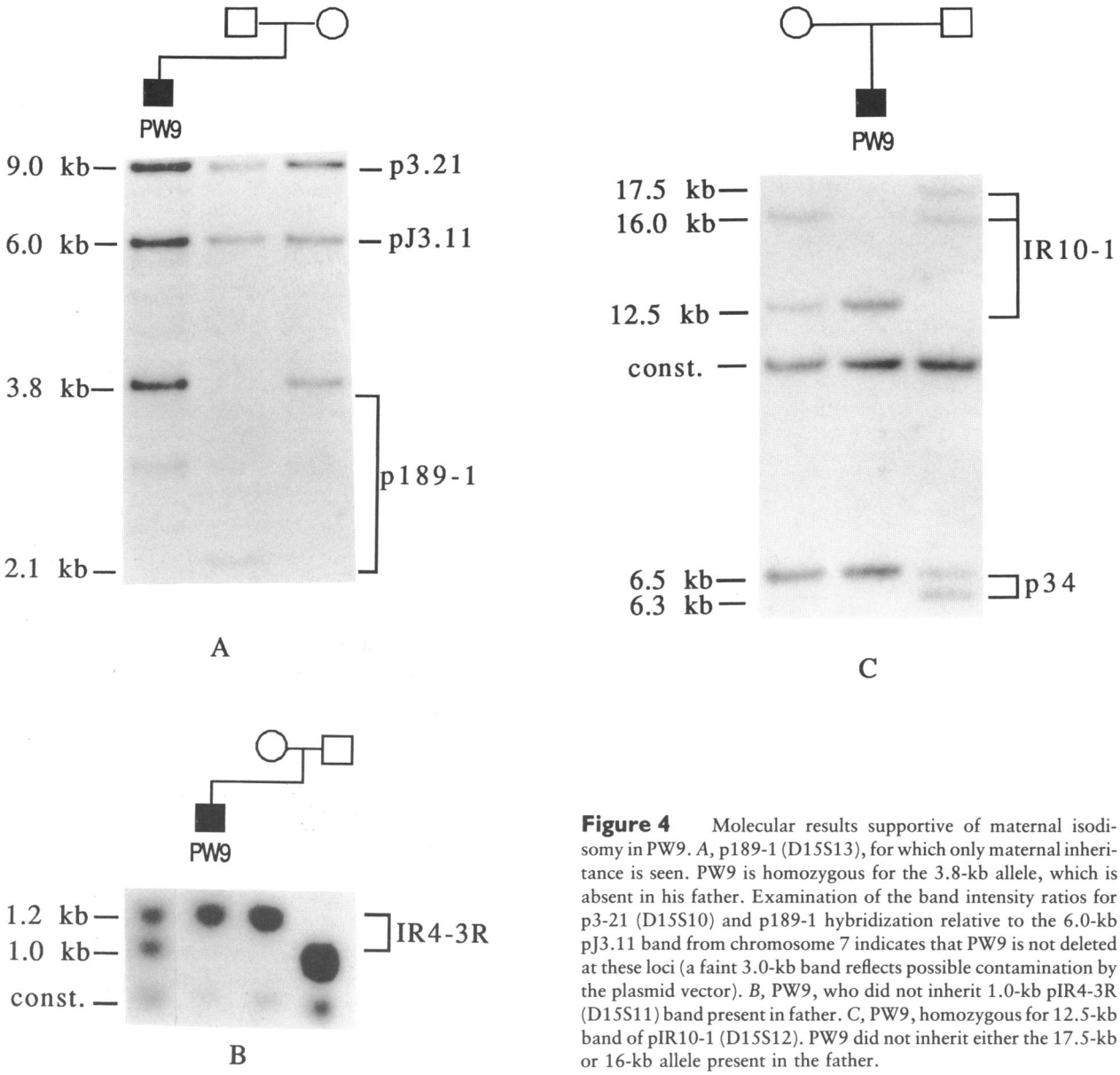

Figure 4 Molecular results supportive of maternal isodisomy in PW9. *A*, p189-1 (D15S13), for which only maternal inheritance is seen. PW9 is homozygous for the 3.8-kb allele, which is absent in his father. Examination of the band intensity ratios for p3-21 (D15S10) and p189-1 hybridization relative to the 6.0-kb pJ3.11 band from chromosome 7 indicates that PW9 is not deleted at these loci (a faint 3.0-kb band reflects possible contamination by the plasmid vector). *B*, PW9, who did not inherit 1.0-kb pIR4-3R (D15S11) band present in father. *C*, PW9, homozygous for 12.5-kb band of pIR10-1 (D15S12). PW9 did not inherit either the 17.5-kb or 16-kb allele present in the father.

(data not shown) for PW23. These results are consistent with isodisomy of the maternal chromosome 15q11-q13 haplotype. Both these patients are, however, heterozygous and identical to their mothers at locus D15S1, recognized by pMS1-14, which maps distally to the PWS region. Heterodisomy is also observed by using the telomeric probe pMS620. It is thus likely that a recombination has occurred between the PWS region and locus D15S1 during meiosis I in the mother. PW9 is mostly typical for PWS but lacks reduced intrauterine activity, did not require gavage feeding during infancy, does not show cryptorchidism, and does not have fair hair (relative to that of his parents). PW23 also showed no reduced intrauterine activity, had only mild neonatal hypotonia, and was above average in height.

In nine patients (PW4, PW29, PW46, PW55, PW56, PW64, PW66, PW70, and PW79), normal maternal and paternal inheritance could be shown. Inheritance of paternal alleles not present in the mother was observed in patient PW29 by using p189-1 and pIR4-3R; in patient PW46 by using probes pIR4-3R

and pMS-620; in patients PW4, PW55, and PW56 by using pMS-620; in patient PW64 by using p189-1 and pCMW-1; in patient PW66 by using pMS1-14; and in patients PW70 and PW79 by using p189-1 (data not shown).

Seven of these patients (PW4, PW29, PW55, PW56, PW64, PW70, and PW79) were independent of the molecular results, found to not fulfill diagnostic criteria for PWS. None of these patients showed the typical history of PWS, as they did not have neonatal hypotonia or feeding problems in infancy. PW55 has been discovered to have a craniopharyngioma of the pituitary gland. As hypothalmic or pituitary dysfunction potentially explain some major features of PWS, this tumor could be the explanation for PWS-like features in this patient.

In two cases—PW66 and PW46—originally classified as atypical PWS, normal maternal and paternal inheritance was seen for chromosome 15, and no molecularly detectable deletion was found. The first patient does, however, have a ring-15 chromosome of paternal origin (as judged by cytogenetics) in all cells; the second patient carries an apparently balanced de novo translocation between chromosome 13 and X (breakpoints at 13q13 and Xq13).

Comparison of Clinical Features

In order to examine homogeneous groups, we only compared the clinical features between deletion and disomy patients. Of 17 deletion patients whose birth weight and term at birth were known, 12 had birth weight below the 10th percentile (as compared with standard tables, based on both term at birth and sex, for birth weight of newborns in Switzerland [Prader et al. 1989]). For only two of seven disomy patients had birth weight below the 10th percentile; however, these differences do not reach statistical significance. The low birth weight of PWS patients seems to be correlated with term at birth, with later-term babies showing the lowest weight percentiles.

Sex ratios among disomy and deletion patients in the present study are significantly different from each other. All seven disomy patients are male ($P = .008$, on the assumption that there is an equal likelihood of obtaining males or females), whereas only nine of 21 deletion patients are male. Other groups, however, have seen equal numbers of male and female disomy patients (R. Nicholls, personal communication).

With means of 27.1 and 30.0 years, respectively, neither maternal nor paternal ages at birth of deletion patients appeared to be increased or decreased; however, the ages of parents of disomy PWS patients were significantly increased relative to those of parents of deletion patients, the mean maternal and paternal ages being 35.6 ($P < .005$, Student's t-test) and 38.7 ($P < .005$), respectively (table 5).

A comparison of additional features in deletion versus disomy patients is given in table 6. Because of small sample sizes (information on each trait was not available for all patients), statistical comparisons cannot be made. However, only a few characteristics seem to show any potential difference between the two groups. The absence of intrauterine activity, commonly noted during pregnancy with a PWS child, is found in 17 of 19 deletion patients, but both isodisomy patients showed normal fetal activity. Seven of 15 deletion patients had to be gavage fed for more than 4 wk, whereas only one of six disomic patients required this. In general, there were no striking differences, in clinical features, between the deletion and nondeletion patients. It will be necessary to study larger numbers of patients to see whether any differences, other than parental ages can be statistically identified.

Discussion

The results presented here are consistent with the hypothesis that most, if not all, PWS patients lack paternal inheritance of a segment of chromosome 15q11.2-q12, with the only possible exception being a clinically atypical patient with a t(13;X).

Of 29 clinically classified PWS patients, 21 (72%) showed molecular deletions. Little variation in the extent of the deletion was observed with the probes used here, with only one patient showing a deletion for some but not all of probes p189-1, pIR10-1, pIR4-3R, p3-21, and p34. All five of these probes have also been found to be deleted in (1) five PWS deletion patients reported by Donlon (1988); (2) three of four PWS

Table 5

Mean ± SD Ages of Parents at Birth of Patient

Patient Group	Mean ± SD Age of (years)	
	Mother	Father
Deletion.....	27.1 ± 5.2 ($N = 20$)	30.0 ± 4.6 ($N = 18$)
Disomy.....	35.6 ± 6.8 ($N = 7$)	38.7 ± 8.4 ($N = 7$)
Nondeletion/ nondisomy ..	29.1 ± 4.9 ($N = 8$)	31.6 ± 2.8 ($N = 8$)

Table 6

Frequencies of Common PWS Features among Disomy and Deletion Patients

Clinical Features	No. with Clinical Feature/Total No.	
	Disomy Patients	Deletion Patients
Reduced intrauterine activity	5/7	17/19
Breech birth	1/1	5/12
Birth weight below 10th percentile	2/7	12/17
Birth length below 10th percentile	1/4	1/12
Neonatal hypotonia	7/7	20/20
Gavage feeding required	4/6	14/18
More than 4 wk gavage feeding required	1/6	7/15
First steps at less than 2 years of age	1/2	10/13
Hyperphagia onset at less than 3 years of age	3/3	11/11
Weight below 97th percentile	3/5	11/21
Height below the third percentile	1/4	10/20
Hand length below the third percentile	2/3	13/15
Foot length below the third percentile	2/3	14/16
Knock-knees	6/6	16/16
Strabismus	3/5	13/16
Hypotonia	6/6	18/18
Mental/motor retardation	7/7	21/21
Hypogenitalism	6/7	16/17
Cryptorchidism	5/7	9/9
Characteristic facies	6/6	19/19
Narrow forehead	6/7	16/16
Frontal upsweep	2/7	7/10
Epicanthal folds	3/5	6/13
Down-slanting fissures	3/7	5/12
Fair hair (relative to parents)	3/4	13/14
Striae	3/6	6/14
Scars from scratching	3/5	11/12
Indifference to pain	3/4	9/11

deletion patients (one of these patients, DON10, is the same as one reported in Donlon 1988) reported by Tantravahi et al. (1989), with the fourth showing a deletion for all probes except pIR10-1; and (3) 13 AS patients (Knoll et al. 1990). This latter study, on AS, showed variation in the extent of the deletion for locus D15S18 (p39), which was not examined in any of the deletion patients reported here.

Our results combined with those of Tantravahi et al. (1989) indicate that only probe p3-21 is common to all deletions. Thus the critical PWS region should lie between the locus detected by pIR10-1 and those detected by p189-1, pIR4-3R, and p34 (the order of these has not yet been firmly established).

There has been one report, by Gregory et al. (1990), in which microdeletions and duplications in PWS were detected with molecular probes; however, Gregory et al. interpreted lack of paternal inheritance to be proof of a deletion, and densitometry was only performed when the families were uninformative for RFLPs. Any uniparental disomy patients would, by this method, seem to be deleted at some loci and not at others in this region. The presence of duplications for p3-21 in three of the eight patients they examined is enigmatic and, as the data were not presented, difficult to evaluate. However, in our experience, several aspects of densitometric analysis can give misleading results. First, if the amount of DNA loaded per lane is not approximately equal, then the test-band: control-band ratio may vary among controls, and it may be important to correct for intensity of each sample (the 2:1 copy ratio will decrease as the intensity of bands increases). Second, comparing the sample with both normal (double-dose) and deleted or heterozygous individuals seems to us to yield more reliable results.

As no patients here or elsewhere have been reported with deletions of these probes who did not present with PWS (when inherited on the paternal chromosome 15) or AS (when originating maternally), molecular analysis should be useful for diagnosing patients during early stages of the syndrome, prior to the onset of all diagnostic features. The deletion in five of these cases was not observed cytogenetically, as the resolution was not high enough either to include or to exclude a deletion. Thus, even these cases are probably not true microdeletions, and they might well show a cytogenetically visible deletion if the cytogenetics could be repeated and if higher resolution could be obtained. However, our experience is that it is much easier to produce a confident diagnosis with molecular probes than with cytogenetic techniques. This difference will clearly vary for different laboratories, depending on their quality for both techniques.

Of the eight nondeletion PWS patients, all seven typical cases were shown to have maternal disomy. Thus, maternal disomy seems to explain all remaining typical PWS patients in whom a deletion was not found. A deletion or disomy of chromosome 15 could not be shown in any of the cases which showed some features of—but did not fulfill diagnostic criteria for—PWS; however, one patient did have a ring-15 chromosome.

As a deletion and maternal disomy have been excluded from the female with an apparently balanced X;13 translocation, one must consider that the translocation could be the cause of the associated pheno-

type. There is a previous report of an 18-mo-old female infant who had an apparently balanced de novo X;13 translocation and whose main features were hypotonia and developmental delay (Hodgson et al. 1986). At 4 years of age, this child was below the third percentile for height but around the 50th percentile for weight, had a strong appetite, and showed severe hypotonia (S. Hodgson and V. Dubowitz, personal communication). A breakpoint at Xq13, which is presumed to be the site of the X-inactivation center (Brown et al. 1991), is common to both cases. It has been suggested that genome imprinting results from the action of dosage-sensitive modifier genes located on the sex chromosomes (Sapienza 1990), and it is possible that such a trans-acting factor on the X chromosome has been disrupted in these patients.

As the ring-15 chromosome in PW66 is of paternal origin, it may be that decreased expression of genes in the PWS region of this chromosome—perhaps by partial inactivation of this region, similar to the "position-effect variegation" seen in *Drosophila* (Spofford 1976)—could explain the phenotype of this patient. Although this patient showed no mosaicism in blood culture, it is conceivable that the ring might have been lost, leading to a deletion of the paternal chromosome 15 genes, in some tissues. Common clinical findings among previously reported patients with ring-15 chromosomes include severe intrauterine and postnatal growth retardation, small hands and feet, male genital hypoplasia, and mild to severe mental retardation (Schinzel 1983). In particular, several patients with ring-15 chromosomes have been reported to have multiple PWS features; these patients include a female with short stature, feeding problems in infancy, hypotonia, and mental retardation (Kousseff 1980); a female with low birth weight, short stature, hypotonia, and mental retardation (Yunis et al. 1981); a female with low birth weight, small hands and feet, hypotonia, and mental retardation (Kiss and Osztovics 1982); a sterile 34-year-old male showing small stature, obesity, and slight mental retardation (Moreau and Teyssier 1982); and a female with low birth weight, short stature, sloping forehead, strabismus, small/short nose, small hands, hypotonia, and mental retardation (Fryns et al. 1986).

The observation that there seems to be very little clinical difference between disomy and deletion patients is intriguing and implies that increased dosage of maternal genes does not compensate in any way for the lost paternal genes. The cases presented here appear to show uniparental disomy for the entire chromosome 15, as is indicated by nonpaternal inheritance of alleles at the telomeric VNTR detected by pMS-620. This implies both (*a*) that imprinting effects due to loss of a paternal segment of chromosome 15 are restricted to the region normally deleted in PWS and (*b*) that maternal disomy for other parts of the chromosome has little or no phenotypic effect.

It should be stressed that heterodisomy and isodisomy are not necessarily mutually exclusive phenomena. They will occur for different parts of the same chromosome pair whenever normal meiotic I recombination occurs prior to the nondisjunction event. In the situation where the nondisjoined chromosomes show reduction to homozygosity of markers nearest to the centromere, the nondisjunction event is inferred to have taken place at meiosis II, whereas heterozygosity of markers closest to the centromere is indicative of a meiotic I nondisjunction event. In patients PW9 and PW23, the chromosome 15 markers examined are homozygous (isodisomic) proximal to the centromere and are heterozygous (heterodisomic) more distally. If no recombination has occurred between the PWS markers and the centromere, then these patients would be the result of a meiosis II nondisjunction event with normal meiotic I recombination.

The nondisjunction event in the mothers of PW1, PW54, PW63, PW77, and PW81 most likely, but not necessarily, occurred in meiosis I, as all the informative markers closest to the centromere were heterozygous. A recombination between the loci recognized by probes pMS1-14 and pMS620, has occurred in PW1, and a recombination between the loci recognized by probes pCMW-1 and pMS-620 has occurred in PW77. The other three heterodisomy cases show heterozygosity at all informative markers tested.

Meiotic I nondisjunction events in sex chromosome aberrations are associated with increased maternal ages, whereas meiotic II nondisjunction events are not (Jacobs 1989; May et al. 1990). The maternal and paternal ages for the disomy patients in the present study were significantly increased. A paternal increase may simply be due to the correlation between maternal and paternal ages. However, for one patient (PW9), who, as mentioned above, is suggestive of a maternal meiotic II nondisjunction event, the maternal and paternal ages at birth of the patient were 41 and 42 years, respectively. This observation can be explained if a recombination has occurred between the PWS region and the centromere, making it really a result of a meiotic I nondisjunction. Alternatively, it is possible that meiotic II nondisjunction for autosomal chromo-

somes has a maternal age effect. Last, there may be a paternal age increase associated with nondisjunction, as, of course, loss of the paternal chromosome must occur for uniparental disomy to be observed.

If one assumes a PWS frequency of 1/20,000 and uses our observed frequency of disomy (i.e., seven of 29 patients clinically diagnosable as PWS), then the estimated frequency of maternal disomy is approximately 1.2×10^{-5}. In his initial proposal on the theoretical possibility of uniparental disomy, Engel (1980) estimated that chromosome 15 uniparental disomy, maternal and paternal together (with equal frequencies of each), should be about 9×10^{-6}. Given the inaccuracies of both estimates, they are not incompatible. This latter estimate was made by assuming both that hyperploidy in males and females is equal and that hypoploidy should occur at the same frequency as hyperploidy. However, approximately 95% of chromosome 16, 18, and 21 trisomies, which have been studied using molecular probes, are maternal in origin (Kupke and Muller 1989; Antonarakis et al., 1991; Hassold et al. 1991). A similar sex bias is likely to exist for chromosome 15. In male gametes, hypoploidy may be more common than hyperploidy, as evidenced by studies both of sperm cells (Martin and Rademaker 1990) and of parental origin in 45,X individuals (Jacobs et al. 1990). These observations together would imply that maternal disomy should in general be more frequent than paternal disomy. A proportion of disomic individuals are also likely to have arisen by early loss of a chromosome in initially trisomic individuals (most of whom would have an extra chromosome from the mother). This may thus explain the observation that paternal disomy of chromosome 15 is found less frequently in AS (Knoll et al. 1991; Malcolm et al. 1991) than is maternal disomy in PWS.

In summary, either a 15q11.2-q12 deletion inherited from the father or maternal disomy of the entire chromosome 15 appears to explain the majority of PWS cases and all of those with very typical features. The striking increase in parental ages is consistent with the hypothesis that disomy patients normally result either from two independent nondisjunction events, one in each parent, or from an initial trisomy. The lack of features which distinguish disomy from deletion patients implies that chromosome 15 has only one "imprinting center," located within 15q11.2-q12.

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